

## Short Report

Presence of antibodies to *Hantavirus* in rat and human populations of Djibouti

G. Rodier<sup>1,2</sup>, A. K. Soliman<sup>1</sup>, J. Bouloumié<sup>1</sup> and D. Kremer<sup>1</sup> <sup>1</sup>US Naval Medical Research Unit No.3, Cairo, Egypt; <sup>2</sup>International Health Program, University of Maryland School of Medicine, Baltimore, Maryland, USA; <sup>3</sup>Djibouti Ministry of Health, Republic of Djibouti

The diagnosis of human haemorrhagic fever with renal syndrome (HFRS) has not been recorded in the Horn of Africa. Since the identification of a virus as agent of HFRS (LEE *et al.*, 1978), several serotypes of this virus have been described as members of a new genus of viruses, *Hantavirus*, within the family Bunyaviridae (LEDUC *et al.*, 1986). Several human cases of HFRS have been reported in Central Africa (COULAUD *et al.*, 1987).

To assess the potential threat of human disease from *Hantavirus* infection in the Horn of Africa, rats were captured and studied in the city of Djibouti during October 1991. Rodents and other small mammals were live-trapped indoors and outdoors from several regions of the city, anaesthetized, and specifically identified. Sera from blood collected by cardiac puncture were preserved by freezing at -80°C and then tested at US Naval Medical Research Unit No.3 (NAMRU-3) by immunofluorescent antibody (IFA) assay.

Antigen spot slides prepared from E-6 Vero cells infected with the Hantaan serotype of Korean haemorrhagic fever (KHF) virus were used for testing sera for Hantaan antibody. Sera were diluted 1:10, 1:20, 1:40 and 1:80 in phosphate-buffered saline; then, for each dilution, one drop was placed on the ringed antigen area of the slides. Slides were incubated at 37°C for 30 min, then rinsed in distilled water and dried. Affinity-purified anti-species immunoglobulin G (Ig G) conjugated with fluorescein isothiocyanate (K & P Laboratories, Rockville, Maryland, USA) was diluted at 1:30 and a drop was placed on each of the ringed antigen areas. Slides were incubated at 37°C in a moist chamber for another 30 min, washed as before, mounted with buffered glycerol (pH 8.6) and examined with a fluorescence microscope at 400-600 magnification. For a sample diluted 1:40, bright apple green colour associated with antigen cells was considered to indicate antibody positivity. Positive and negative control sera were included with each test.

One hundred and seventy-three small mammals were captured, with 57.8% from the port area and *R. norvegicus* the primary species (78.5%). The overall *Hanta-*

Table. Sera reactive by immunofluorescence for antibodies against *Hantavirus* in rodent and human sera

Serum	No.	Highest dilution for reactivity*			
		1:10	1:20	1:40	1:80
<i>R. norvegicus</i>	108	6	3	2	7
Other rodents	65	0	-	-	-
Djiboutian army	198	20	7	3	4
Djiboutian navy	14	2	0	-	-

\*Positive test requires reactivity at a dilution  $\geq 1:40$

Address for correspondence: Commanding Officer, U.S. Naval Medical Research Unit No.3, PSC 452, Box 5000, FPO AE 09835-0007, USA; Attn: Dr G. Rodier, Epidemiology Branch.

Address for offprint requests: Research Publication Branch, US Naval Medical Research Unit No.3, PSC 452, Box 5000, FPO AE 09835-0007, USA.

virus positivity rate was 5.2%. All 49 *Rattus rattus*, 9 *Crocidura somalica* (shrews), and 7 *Mus musculus* sera were negative for *Hantavirus* antibodies. Only 9 of the 108 *R. norvegicus* sera were positive for antibodies to *Hantavirus* (prevalence 8.3%) (Table). All *Hantavirus* reactive sera (from 1:10 to 1:80 dilution) were from *R. norvegicus* caught in the port area. There was no statistically significant association between Hantaan positivity and sex, age (adult or young), or indoor or outdoor collection.

In order to assess the prevalence of *Hantavirus* infection in the human population of Djibouti, 212 human sera were tested. These sera had been collected in May 1991 from Djiboutian military personnel enrolled by consecutive sampling, without selection criteria, at the local military clinic during a human immunodeficiency virus survey. The mean age was 33.2 years (range 20-54 years). Thirty-six of 212 human sera (16%) were reactive for IgG to *Hantavirus* at 1:10 dilution, but only 7 (3.3%) were considered positive (reactive at dilutions  $\geq 1:40$ ). There was no statistically significant association between location of work or residence and antibodies to *Hantavirus*. In particular, naval personnel working frequently in the port area did not show a higher prevalence of *Hantavirus* infection (Table).

The association between rat seropositivity and the port area contrasts with the absence of correlation among the indigenous human population. This underlines the difficulty in estimating the epidemiology of *Hantavirus* infection. A definite association between *R. norvegicus* and Seoul virus, a *Hantavirus* member known to cause moderate to severe disease in human, has been previously described (CHILDS *et al.*, 1987).

The absence of reports of human cases of HFRS in Djibouti suggests either the predominance of asymptomatic infections or that symptoms of infection such as mild febrile illness are misdiagnosed as common febrile diseases such as malaria. Another hypothesis is the presence of a non-pathogenic Hantaan-related virus, as already suggested elsewhere in Africa (GONZALES *et al.*, 1984, 1989), that is possibly not related with the virus in the rat population.

## Acknowledgements

We thank the officials of the Djiboutian Ministry of Health who encouraged and facilitated this study. Special appreciation goes to Mr Hassan Touhami and Fathi Aboulfadl. Epidemiology Branch, NAMRU-3, and Mr Mohamed Abro Moussa and Mr Djilani Girud Dabaleh, Service d'Hygiène, Djibouti. Thanks also go to Dr Rémi Jeannin, Dr Jean-Jacques Lataillade, and Dr Dominique Pineau from Service de Santé de l'Armée Nationale, Djibouti.

This study was supported by the Naval Medical Research and Development Command, Naval Medical Command, National Capital Region, Bethesda, MD 20814, USA, Work Unit No. 3M463105.H29.AA.335. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy, the Government of the United States, the Department of Defense, the Djibouti Ministry of Health, or the World Health Organization.

## References

- Childs, J. E., Korch, G. W., Glass, G. E., Leduc J. W. & Shah, K. V. (1987). Epizootiology of *Hantavirus* infections in Baltimore: isolation of a virus from Norway rats, and characteristics of infected rat populations. *American Journal of Epidemiology*, 126, 55-68.
- Coulaud, X., Chouaib, E., Georges, A. J., Rollin, P. & Gonzales, J. P. (1987). First human case of haemorrhagic fever with renal syndrome in the Central African Republic. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 81, 686.
- Gonzales, J. P., McCormick, J. B., Baudon, D., Gautun, J. P., Meunier, D. Y., Dournon, E. & Georges, A. J. (1984). Serological evidence for Hantaan-related virus in Africa. *Lancet*, ii, 1036-1037.
- Gonzales, J. P., Josse, R., Johnson E. D., Merlin, M., Georges A. J., Abandja, A. J., Danyold, M., Delaporte, E., Dupont, A. & Ghogomu, A. (1989). Antibody prevalence against he-

- morrhagic fever viruses in randomized representative Central African populations. *Research in Virology*, **140**, 319-331.
- Leduc, J. W., Smith, G. A., Childs, J. E., Pinheiro, F. P., Maitztegui, J. I., Niklasson, B., Antoniadis, A., Robinson, D. M., Khin, M., Shortridge, K. F., Wooster, M. T., Elwell, M. R., Ilberon, P. L. T., Koech, D., Rosa, E. S. T. & Rosen, L. (1986). Global survey of antibody to Hantaan-related viruses among peridomestic rodents. *Bulletin of the World Health Organization*, **64**, 139-144.
- Lee, H. W., Lee, P. W. & Johnson, K. M. (1978). Isolation of the etiological agent of Korean hemorrhagic fever. *Journal of Infectious Diseases*, **137**, 298-308.

*Received 11 March 1992; revised 11 June 1992; accepted for publication 11 June 1992*

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188																									
<small>Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.</small>																												
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 11 MAR 1992	3. REPORT TYPE AND DATES COVERED																										
4. TITLE AND SUBTITLE Presence of Antibodies to <u>Hantavirus</u> in Rat and Human Populations of Djibouti		5. FUNDING NUMBERS PE- 63105A WU- 3M463105H29.AA.335																										
6. AUTHOR(S) Rodier, G., Soliman, A.K., Bouloumie, J. and Kremer, D.																												
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) U.S. Naval Medical Research Unit No. 3 PSC 452, Box 5000 FPO AE 09835-0007		8. PERFORMING ORGANIZATION REPORT NUMBER  26/93																										
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Naval Medical Research and Development Command, National Naval Medical Center Building 1, Tower 12 Bethesda, MD 20889-5044		10. SPONSORING / MONITORING AGENCY REPORT NUMBER																										
11. SUPPLEMENTARY NOTES  Published in: Trans. R. Soc. Trop. Med. Hyg., 87:160-161, 1993; Acc. No. 1748.																												
12a. DISTRIBUTION / AVAILABILITY STATEMENT  Approved for public release; Distribution is unlimited.		12b. DISTRIBUTION CODE																										
13. ABSTRACT (Maximum 200 words)  Please see attached.		<table border="1"> <tr> <td colspan="2">Accession For</td> </tr> <tr> <td>NTIS</td> <td><input checked="" type="checkbox"/></td> </tr> <tr> <td>CRA&amp;I</td> <td><input checked="" type="checkbox"/></td> </tr> <tr> <td>DTIC</td> <td><input checked="" type="checkbox"/></td> </tr> <tr> <td>TAB</td> <td><input checked="" type="checkbox"/></td> </tr> <tr> <td>Unannounced</td> <td><input type="checkbox"/></td> </tr> <tr> <td>Justification</td> <td></td> </tr> <tr> <td colspan="2">By _____</td> </tr> <tr> <td colspan="2">Distribution / _____</td> </tr> <tr> <td colspan="2">Availability Codes</td> </tr> <tr> <td>Dist</td> <td>Avail and/or Special</td> </tr> <tr> <td colspan="2">A-1 20</td> </tr> </table>			Accession For		NTIS	<input checked="" type="checkbox"/>	CRA&I	<input checked="" type="checkbox"/>	DTIC	<input checked="" type="checkbox"/>	TAB	<input checked="" type="checkbox"/>	Unannounced	<input type="checkbox"/>	Justification		By _____		Distribution / _____		Availability Codes		Dist	Avail and/or Special	A-1 20	
Accession For																												
NTIS	<input checked="" type="checkbox"/>																											
CRA&I	<input checked="" type="checkbox"/>																											
DTIC	<input checked="" type="checkbox"/>																											
TAB	<input checked="" type="checkbox"/>																											
Unannounced	<input type="checkbox"/>																											
Justification																												
By _____																												
Distribution / _____																												
Availability Codes																												
Dist	Avail and/or Special																											
A-1 20																												
14. SUBJECT TERMS Antibodies; Hantavirus; Human hemorrhagic fever; Renal syndrome; Rats; Humans; Djibouti.		15. NUMBER OF PAGES 2																										
		16. PRICE CODE																										
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT																									